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Microarray Analysis of Human Blood During Electroconvulsive Therapy

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Introduction: Electroconvulsive therapy (ECT) is currently regarded as a significant treatment option for intractable psychiatric disorders, such as catatonic schizophrenia or treatment-resistant depression; however, the underlying molecular mechanism for its therapeutic effect remains obscure.

Methods: Employing microarray analysis (Human Genome U133 Plus 2.0 Array; Affymetrix, United States) of cDNA derived from the peripheral blood of patients with catatonic schizophrenia (n = 5), we detected a significant change in 145 genes (0.68%) before and after modified ECT (mECT). Moreover, we performed quantitative polymerase chain reaction validation of genes that had previously been suggested to be functionally related to schizophrenia.

Results: Of 4 genes examined (*AKT3*, *TCF7*, *PPP3R1*, and *GADD45B*), only *TCF7* was increased during the mECT procedure ($P = 0.0025$).

Discussion: This study describes the first attempt to uncover the molecular mechanism of mECT using a microarray assay of mRNA derived from peripheral blood, and our results suggest that the TCF family may play a role in the functional mechanism of mECT.

Key Words: modified electroconvulsive therapy, mECT, microarray, catatonic schizophrenia, quantitative polymerase chain reaction, qPCR, AKT3, TCF7, PPP3R1, GADD45B, mRNA, gene expression

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Electroconvulsive therapy (ECT) is currently regarded as a valuable treatment option for intractable psychiatric disorders. In particular, in the case of drug-resistant depression[1,2] or catatonic schizophrenia,[3,4] ECT can be very effective if the appropriate technique is used. Although the underlying molecular mechanism for this therapy remains unclear, some key findings have been reported thus far.[5,6] Briefly, in the context of animal models, electroconvulsive shock (ECS) induces the activation of *N*-methyl-D-aspartate[7,8] (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid–related receptors (AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid[9]) followed by increased hippocampal neurogenesis. These previous findings were based on assessments of biological samples treated with ECT (ECS); both humans and rodents have been studied.

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For example, the expression of mRNA of neuropeptide Y (NPY) at rat dorsal spinal cords,[10] hippocampus,[11,12] dentate gyrus, and piriform cortex[13] was increased, whereas its receptors (NPY1r, NPY2r, and NPY5r) were decreased.[12]

Previous analyses using quantitative polymerase chain reaction (qPCR) have been driven primarily by functional interest in a selected gene, and a comprehensive analysis of gene expression in human samples has not been performed thus far. The adoption of microarray technology for analysis of biological samples shows a strong advantage over qPCR because of the overwhelming abundance of genes that may be analyzed. Moreover, the latter technology has a greater possibility of detecting genes that are actually involved because the technique is not restricted by functional hypotheses. When samples are analyzed appropriately, microarray technology provides novel insight on biological change. Using microarrays, we sought to evaluate changes in mRNA gene expression in peripheral blood samples between pre-ECT and post-ECT in real-world clinical practice.

METHODS

Patient Recruitment

Among patients with a diagnosis of schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorders IV Text Revision[14] and a prescription history of 2 or more types of antipsychotics at maximal dose for more than 4 weeks that resulted in insufficient treatment effect, we recruited only those treated with modified ECT (mECT; ie, ECT under general anesthesia) at Osaka Medical College Hospital. Five patients with schizophrenia with catatonic subtype were recruited for microarray analysis. Notably, we employed only the patients with more than 20% improvement in symptoms based on the The Positive and Negative Syndrome Scale[15] (PANSS) for schizophrenia. The demographics of this sample including their symptom assessments are presented in Table 1.

Ethical Consideration

This study was approved by the ethics committee of the Osaka Medical College. Written, informed consent for biological assessment was provided by all participants after receiving an oral explanation and viewing a documentary. For this current study, surrogate approval for inclusion in the study was not accepted.

Medication

Before starting mECT, we had basically stopped both benzodiazepine anxiolytic and anticonvulsant, and the content of antipsychotic drug was changed after the mECT because previous medication was not effective enough for the treatment.

TABLE 1. Demographics of the Sample Analyzed (Including PANSS Changes)

Disorder	Age	Sex	Subtype	PANSS Before mECT	PANSS After mECT
Schizophrenia	51	Female	Catatonic	172	81
Schizophrenia	63	Female	Catatonic	148	77
Schizophrenia	63	Female	Catatonic	170	75
Schizophrenia	62	Male	Catatonic	149	102
Schizophrenia	54	Male	Catatonic	120	89

mECT Procedure and Sample Treatment

Modified electroconvulsive therapy was administered 10 times in total, at a frequency of twice per week under general anesthesia. Using Thymatron (Somatics LLC, Lake Bluff, Ill), the patients were evoked using bilateral electrical stimulation with a pulse wave. We set the initial quantity of electricity using the half-age method (quantity of electricity at half age[16]), and the stimulation dose was increased 1.5-fold if an effective convulsive seizure was not obtained. We used propofol (intravenous, 1.0 mg/kg) to induce general anesthesia, and suxamethonium (0.8 mg/kg) was used as a muscle relaxant. Modified electroconvulsive therapy was performed under hospitalization to minimize the effect of sleep, dietary habits, the presence or absence of smoking, or medications; we did not change medications during the mECT. Peripheral blood (3 mL) was extracted at approximately 10:00 AM 1 day before the ECT (day 0) and on the day after the sixth ECT session (day 19). The sample was injected into PAXgene blood RNA tubes (PreAnalytix, England), RNA was then extracted using an mRNA Isolation Kit for Blood (Roche Diagnostics, Switzerland), and complementary DNA was subsequently synthesized.

Microarray Analysis

The collected RNA quality, approximate yield, and A260/280 were evaluated using a spectrophotometer (NanoDrop 1000; Thermo Scientific, United States). Because all of the collected samples passed the quality control stage, microarray analysis was performed using a Human Genome U133 Plus 2.0 Array (Affymetrix, United States).

Quantitative Polymerase Chain Reaction

Peripheral blood samples were injected into PAXgene Blood RNA tubes. For analysis, RNA was extracted using the mRNA Isolation Kit for Blood and complementary DNA was subsequently synthesized, similar to the microarray procedure. The LightCycler system (Roche Diagnostics, Switzerland) was employed to determine mRNA expression. The mRNA expression in each sample was normalized to the expression level of a housekeeping gene (*GAPDH*). The primers and probes are described in Table 2, and the PCR condition has followed the following standard procedures: (preparation for hot start) 95°C; (PCR, 40 cycles) 95°C in 15 seconds and 60°C in 30 seconds; and (cooling, 1 cycle) 40°C in 30 seconds.

Statistics Procedure

All statistical analyses were performed using SPSS Statistics 22 (IBM, United States). Paired *t* test was used for the comparison on the microarray analysis, whereas analysis of variance was applied on the qPCR data.

RESULTS

On the basis of the microarray analysis, 21,388 transcription substitutions were revealed to be present within 47,000 substitutions in 10 microarray assessments. Subsequently, *t* tests comparing pre- and post-mECT expressions uncovered 145 gene substitutions for further assessment ($P < 0.01$). Of these 145 genes, *AKT3* (v-akt murine thymoma viral oncogene homolog 3; gene ID, 10000), *TCF7* (transcription factor 7; gene ID, 6932), *PPP3R1* (protein phosphatase 3, regulatory subunit B, alpha, also known as calcineurin; gene ID, 5534), and *GADD45B* (growth arrest and DNA damage inducible, beta; gene ID, 4616) were selected, on the basis of their functional involvement in schizophrenia, for further validation by qPCR (Table 3 and details are described in the Discussion section). In addition to the samples obtained the day before the first ECT session (day 0) and the day after the sixth ECT session (day 17), we also evaluated gene expression in the samples from the day after the third ECT session

TABLE 2. Probes and Primers Used for the 4 Selected Genes

Akt3	Forward primer	5′-GCAAACGCAGCTCCAACTTA-3′
	Reverse primer	5′-GTGCATGATTCTCATCAGCCTGTA-3′
	Hydrosis probe	FAM-TGCAGTTACTTTTGACCCATGTGCAAGGAT-TAMRA
Gadd45b	Forward primer	5′-TGCATACGAGAGACTTGGTTGA-3′
	Reverse primer	5′-ATGGGTACAGAGCAACTTCAG-3′
	Hydrosis probe	FAM-CTTGGTTGGTCCTTGTCTGCACCCT-TAMRA
PPP3R1	Forward primer	5′-TCCACCCAGCCCATTTCA-3′
	Reverse primer	5′-CGATCAGTACTCTCACCGTTTG-3′
	Hydrosis probe	FAM-CCAGATAAAAGGGGTCTCTGCGTCTGTAG-TAMRA
TCF7	Forward primer	5′-CGTTCCTTCCGATCAGT-3′
	Reverse primer	5′-AGGGCTAGTAGGCAGTTCTGTG-3′
	Hydrosis probe	FAM-CAGCAGATGGTATGAGGGTGAGTCCTGG-TAMRA

Primers used for qPCR amplification of the selected probes.

TABLE 3. The Fold Change in Expression of the 4 Selected Genes Using Microarray Analysis

Gene Symbol	Function	Regulation	*P*	Fold Change
Gadd45b	DNA demethylation	Down	0.00019	1.23
TCF (*TCF7*)	Lymphocyte differentiation	Up	0.00551	1.61
CN (*PPP3R1*)	Calcium ion binding Calmodulin binding	Up	0.00698	1.35
Akt (*Akt3*)	Regulation of cell signaling	Down	0.0099	1.2

Statistical procedure: paired *t* test was applied for the data.

(day 7). The time-dependent changes in mRNA expression levels of the 4 selected genes are shown in Figure 1, and qPCR validated the microarray findings for the *TCF7* gene ($F = 7.04$, $P = 0.0095$). Post hoc analysis revealed the significant difference on the comparison of before the third ECT ($P = 0.037$) and before the sixth ECT ($P = 0.011$) on *TCF7* gene expression.

DISCUSSION

It is important to investigate the mechanisms underlying ECT stimulation of the human brain. In the current study, we used peripheral blood to compare changes in gene expression between pre- and post-ECT. To avoid a hypothesis-driven approach, microarray technology was adopted for primary screening and this design enabled us to reduce the list of enormous causal genes to practical numbers. Of note, only 0.68% (145/21388) substitutions were revealed at the $P < 0.01$ level of significance. Among these genes of interest, further assessment employing qPCR confirmed 4 valuable candidates, *AKT*,[17] *TCF7*,[18,19] calcineurin,[20] and *GADD45B*,[21] which are currently regarded as being involved in the etiology of psychiatric disorders. On the basis of qPCR validation, only *TCF7* demonstrated increased expression between the introduction of mECT and the sixth stimulation.

With regard to our significant finding for *TCF7*, one suggested that mechanism for ECT is associated with increased NMDA receptor function or neurogenesis. TCF-family genes including *TCF7* comprise *TCF7/TCF1*, *TCF7L1/TCF3*, *TCF7L2/TCF4*, and *LEF1*. TCF-family genes regulate one another, and

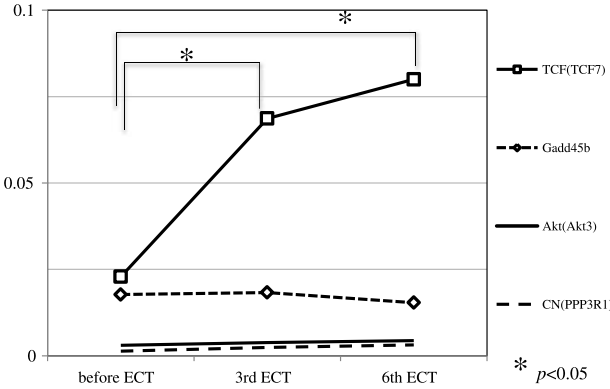

FIGURE 1. Time-dependent mRNA changes in the expression of 4 selected genes (*GADD45B*, *TCF7*, *PPP3R1*, and *AKT3*) using qPCR. Analysis of variance was applied for each gene, and a significant difference only on *TCF7* was found ($F = 7.04$, $P = 0.0095$). The comparison between 2 points, such as before the third ECT and sixth ECT indicated the significant difference at $P < 0.05$ level (post hoc analysis by Tukey method). *x* axis: time course, before ECT (day 0), third ECT (day 7), and sixth ECT (day 17). *y* axis: the average of mRNA expression of 5 cases normalized by *GAPDH* expression.

TCF7 is known to suppress the function of *TCF7L2*.[22] Moreover, through the canonical Wnt/β-catenin signaling pathway, TCF-family genes enter the nucleus with β-catenin to act as transcription factors.[23] Approximately 100 types of genes have been identified as transcriptional targets of Wnt and are therefore referred to as Wnt target genes. Wnt target genes are vital for the initiation of neurogenesis, and TCF-family transcription factors in particular are known to directly regulate neurogenesis.[24] In addition, because NMDA receptor activation induces increased levels of cytoplasmic β-catenin based on the actions of calpain,[25] it is suspected that the mechanisms underlying mECT involve this cascade, such as the activation of NMDA receptors and increasing cytoplasmic β-catenin levels. TCF-family genes then enter the nucleus with β-catenin to induce neurogenesis.

The data presented here should be interpreted with caution. First, because of ethical considerations, our analysis was limited to peripheral blood. Therefore, inevitable limitations exist, such as (1) the lack of any conclusive correlation between blood and cerebral spinal fluid, (2) the invasiveness of the blood draw, which may have affected the procedure itself, and (3) the lack of strict control on oral medication around mECT, which has a potential effect on the quantity of mRNA gene expression. In addition, it is reasonable to suspect the credibility of microarray technology when analyzing peripheral blood samples; thus, we validated the data using qPCR. Although our sample size was limited, this validation step provided a new perspective to our microarray data.

Although the *TCF7* (gene ID, 6932; Chr, 5) gene with functional association to schizophrenia is *TCF7L2* (gene ID, 6934; Chr, 10, also known as *TCF4*), *TCF7L2* belongs to a subfamily of TCF7-like high-mobility group box-containing transcription factors. This gene is involved in the homeostasis of blood sugar, and a genome variant of this gene increases the risk of type 2 diabetes.[26,27] In addition, a large-scale European study of schizophrenia cases (n = 4089) showed a genetic prevalence of the T allele of rs7903146 in the *TCF7L2* gene (original sample set, 410 Danish patients with schizophrenia [$P = 0.0052$]; replication sample set, multinational European patients with schizophrenia [$P = 0.033$]).[18] Furthermore, a *Tcf7l2* knockout mouse also showed anxiety- and fear-related behavioral patterns.[28] Using open field and light/dark box tests, we observed an anxiety-like phenotype comprising decreased time spent in the center of the arena in the light compartment in $Tcf7l2^{+/-}$ mice. In addition, $Tcf7l2^{+/-}$ mice displayed increased freezing behavior both during context and tone stimulation. Although prepulse inhibition, which is a well-known schizophrenia endophenotype, was not decreased in $Tcf7l2^{+/-}$ mice, this gene is suspected to be involved in the alteration of behavior. Our finding on *TCF7* (not implicated in schizophrenia) has an indirect effect of ECT through the action of *TCF7L2* (implicated in schizophrenia) because these 2 genes are clearly different, although they are regulating each other.

A Utah University group led by Dr Light has reported the altered mRNA gene expression of leukocyte after mECT. Nine

target genes, such as *ADRA2A*, *ASIC1*, *AISC3*, *DBI*, *DRD4*, *IL-10*, *NR3C1*, *SULT1A1*, and *VEGFA*, were examined by quantitative real-time-PCR at 22 patients with refractory depression.[29] The design was similar to the current work in terms of the analysis of the peripheral blood sample, although microarray was initially used on our work. As for the array analysis on rat brain, a custom microarray chip (645 genes related to neurotrophic growth factor signaling, neurotransmitter signaling, angiogenesis, vasodilation, and transcription factors) revealed their involvement in acute or chronic electroconvulsive seizure.[30] Another group using the Affymetrics U34 high-density oligonucleotide microarrays has detected 120 genes with altered mRNA expression after ECS at rodent brain.[31] A pathway analysis on their data pointed out the involvement of multiple genes in brain-derived neurotrophic factor—mitogen-activated protein kinase-cAMP-cAMP response element-binding protein pathway, whereas our current data also suggested the involvement of a part of the pathway (mitogen-activated protein kinase signaling pathway). However, it is supposed that no individual gene within 145 genes ($P < 0.01$) on our data has been overlapped with 120 genes reported by Altar et al. It is due to the inconsistency of (1) species (rodents and humans), (2) specimen (brain and blood), and(3) platform (Affymetrics U34 Rat Genome GeneChip being capable of detecting less than 8800 genes and Human Genome U133 Plus 2.0 Array approximately for 47,000 transcripts). Despite their discrepancy, it is noteworthy to point out the existence of 1 overlapped pathway (brain-derived neurotrophic factor—mitogen-activated protein kinase- cAMP-cAMP response element-binding protein pathway) between 2 reports.

Our study potentially uncovers the etiological mechanism of electrical stimulation in the human brain. Although careful research is required to verify the result of our screening, our current work suggests that the TCF family is involved in the molecular mechanism underlying mECT and the etiology of schizophrenia.

REFERENCES

1. Lisanby SH. Electroconvulsive therapy for depression. *N Engl J Med*. 2007;357:1939–1945.
2. Kellner CH, Greenberg RM, Murrough JW, et al. ECT in treatment-resistant depression. *Am J Psychiatry*. 2012;169:1238–1244.
3. Zervas IM, Theleritis C, Soldatos CR. Using ECT in schizophrenia: a review from a clinical perspective. *World J Biol Psychiatry*. 2012;13:96–105.
4. Pompili M, Lester D, Dominici G, et al. Indications for electroconvulsive treatment in schizophrenia: a systematic review. *Schizophr Res*. 2013;146:1–9.
5. Bolwig TG. How does electroconvulsive therapy work? Theories on its mechanism. *Can J Psychiatry*. 2011;56:13–18.
6. Fochtmann LJ. Genetic approaches to the neurobiology of electroconvulsive therapy. *J ECT*. 1998;14:206–219.
7. Salter MW, Kalia LV. Src kinases: a hub for NMDA receptor regulation. *Nat Rev Neurosci*. 2004;5:317–328.
8. Andrade C, Singh NM, Thyagarajan S, et al. Possible glutamatergic and lipid signalling mechanisms in ECT-induced retrograde amnesia: experimental evidence for involvement of COX-2, and review of literature. *J Psychiatr Res*. 2008;42:837–850.
9. Naylor P, Stewart CA, Wright SR, et al. Repeated ECS induces GluR1 mRNA but not NMDAR1A-G mRNA in the rat hippocampus. *Brain Res Mol Brain Res*. 1996;35:349–353.
10. Okabe T, Sato C, Sakamoto A. Changes in neuropeptide Y gene expression in the spinal cord of chronic constrictive injury model rats after electroconvulsive stimulation. *Biomed Res*. 2010;31:287–292.
11. Zachrisson O, Mathé AA, Stenfors C, et al. Limbic effects of repeated electroconvulsive stimulation on neuropeptide Y and somatostatin mRNA expression in the rat brain. *Brain Res Mol Brain Res*. 1995;31:71–85.
12. Dyrvig M, Christiansen SH, Woldbye DP, et al. Temporal gene expression profile after acute electroconvulsive stimulation in the rat. *Gene*. 2014;539:8–14.
13. Mikkelsen JD, Woldbye DP. Accumulated increase in neuropeptide Y and somatostatin gene expression of the rat in response to repeated electroconvulsive stimulation. *J Psychiatr Res*. 2006;40:153–159.
14. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Text Revision*. 4th ed. American Psychiatric Association; 2000.
15. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull*. 1987;13:261–276.
16. Petrides G, Fink M. The "half-age" stimulation strategy for ECT dosing. *Convuls Ther*. 1996;12:138–146.
17. Balog Z, Kiss I, Kéri S. Five new schizophrenia loci may converge on the same cellular mechanism: the AKT pathway. *Am J Psychiatry*. 2012;169:335.
18. Hansen T, Ingason A, Djurovic S, et al. At-risk variant in TCF7L2 for type II diabetes increases risk of schizophrenia. *Biol Psychiatry*. 2011;70:59–63.
19. Alkelai A, Greenbaum L, Lupoli S, et al. Association of the type 2 diabetes mellitus susceptibility gene, TCF7L2, with schizophrenia in an Arab-Israeli family sample. *PLoS One*. 2012;7:e29228.
20. Liu YL, Fann CS, Liu CM, et al. More evidence supports the association of PPP3CC with schizophrenia. *Mol Psychiatry*. 2007;12:966–974.
21. Ma DK, Jang MH, Guo JU, et al. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. *Science*. 2009;323:1074–1077.
22. Roose J, Huls G, van Beest M, et al. Synergy between tumor suppressor APC and the beta-catenin-Tcf4 target Tcf1. *Science*. 1999;285:1923–1926.
23. Wisniewska MB. Physiological role of beta-catenin/TCF signaling in neurons of the adult brain. *Neurochem Res*. 2013;38:1144–1155.
24. Sokol SY. Wnt signaling through T-cell factor phosphorylation. *Cell Res*. 2011;21:1002–1012.
25. Abe K, Takeichi M. NMDA-receptor activation induces calpain-mediated beta-catenin cleavages for triggering gene expression. *Neuron*. 2007;53:387–397.
26. Qu HQ, Polychronakos C. The TCF7L2 locus and type 1 diabetes. *BMC Med Genet*. 2007;8:51.
27. Helgason A, Pálsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet*. 2007;39:218–225.
28. Savic D, Distler MG, Sokoloff G, et al. Modulation of Tcf7l2 expression alters behavior in mice. *PLoS One*. 2011;6:e26897.
29. Iacob E, Tadler SC, Light KC, et al. Leukocyte gene expression in patients with medication refractory depression before and after treatment with ECT or isoflurane anesthesia: a pilot study. *Depress Res Treat*. 2014;2014:582380.
30. Newton SS, Collier EF, Hunsberger J, et al. Gene profile of electroconvulsive seizures: induction of neurotrophic and angiogenic factors. *J Neurosci*. 2003;23:10841–10851.
31. Altar CA, Laeng P, Jurata LW, et al. Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. *J Neurosci*. 2004;24:2667–2677.